Comparison of transfusion with DCLHb or pRBCs for treatment of intraoperative anemia in sheep

LUIZ A. VANE, J. SEAN FUNSTON, ROBERT KIRSCHNER, DON HARPER, DONALD J. DEYO, DANIEL L. TRABER, LILLIAN L. TRABER, AND GEORGE C. KRAMER
Resuscitation Research Laboratories, Departments of Anesthesiology and Physiology, University of Texas Medical Branch, Galveston, Texas 77555-0801

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Vane, Luiz A., J. Sean Funston, Robert Kirschner, Don Harper, Donald J. Deyo, Daniel L. Traber, Lillian L. Traber, and George C. Kramer. Comparison of transfusion with DCLHb or pRBCs for treatment of intraoperative anemia in sheep. J Appl Physiol 92: 343–353, 2002.—Isoflurane-anesthetized sheep were transfused with packed red blood cells (pRBCs) or diaspirin cross-linked hemoglobin (DCLHb) for treatment of intraoperative hemorrhage. A rapid 15-min hemorrhage with lactated Ringer (LR) infusion maintained filling pressure at baseline and reduced blood hemoglobin (Hb) to ~5 g/dl. Sheep received 2 g/kg Hb, DCLHb (n = 6), or pRBCs (n = 7); control group received LR alone (n = 6). After 2 h, anesthesia was discontinued; sheep were monitored in the animal intensive care unit for 48 h. DCLHb expanded blood volume more, but increased total blood Hb less, than pRBCs. Lower Hb and increased methemoglobin resulted in lower arterial oxygen content compared with the pRBCs. DCLHb caused pulmonary hypertension (from 13 to 30 mmHg) and elevated filling pressure (from 6 to 15 mmHg). Cardiac outputs (CO) were similar for all groups during anesthesia; however, during recovery CO increased only in the LR and packed pRBCs groups. DCLHb may limit the reflex ability to increase CO after volume expansion. Hemodynamic effects of DCLHb may be exaggerated when infused after large-volume LR hemorrhage; red blood cell substitutes; hemoglobin; fluid resuscitation; shock; packed red blood cells; diaspirin cross-linked hemoglobin

SUBSTANTIAL EFFORTS HAVE BEEN MADE toward the development of an effective transfusion substitute for packed red blood cells (pRBCs) (9, 36). Most efforts have focused on free acellular hemoglobin (Hb) solutions with the Hb molecule modified chemically or genetically to optimize stability, intravascular retention, and a near-normal oxygen Hb dissociation (9, 14, 22, 35). There are large differences between the Hb content of pRBCs and free Hb solutions. A hematocrit (Hct) typical of pRBCs is 60–75, with a corresponding Hb concentration of 20–25 g/dl (22). Most Hb-based red blood cell (RBC) substitutes have a Hb concentration of 10–15%, and such Hb solutions are typically hyperoncotic colloids and expand blood volume more than the volume of the infused solution. For example, a recent study showed that 10% diaspirin cross-linked hemoglobin (DCLHb) expanded blood volume by ~130% of infused volume (7). On the other hand, pRBCs are suspended in a crystalloid anticoagulant saline buffer solution and would be expected to expand blood volume slightly less than the infused volume, because the saline solution would distribute throughout the entire extracellular space (7). Furthermore, most Hb solutions produce additional pharmacological effects such as vasoconstriction and gastrointestinal dysmotility, whereas pRBCs are largely devoid of such effects (12, 13, 21).

The very different Hb concentrations, volume expansion effects, and pharmacological properties of pRBCs compared with Hb-based pRBCs substitutes would be expected to have significant consequences on oxygen-carrying capacity and hemodynamics after transfusion. Despite this expectation, there are few data available on how RBC substitutes compare directly to pRBCs in clinically relevant conditions of normovolemic anemia. Most preclinical studies of RBC substitutes have focused on infusion in normal animals (top loading), exchange transfusion, or resuscitation of hemorrhagic hypovolemia (4, 5, 10, 25, 28). Furthermore, control groups are typically conventional colloids or colloids or whole blood and not the more clinically relevant pRBCs (4, 15, 19). Clinical trials have shown that RBC substitutes can reduce the need for RBC transfusions in some patients; however, direct comparisons between groups have been difficult because of patient variability and varied transfusion doses (8, 17).

In the present study, we compared equal-dose Hb transfusions of pRBCs vs. DCLHb in anesthetized, surgically stressed sheep subjected to a major hemorrhage and a resulting anemia due to blood loss and lactated Ringer (LR) infusion. Sheep were not allowed to go into hypovolemic shock during the hemorrhage, because LR was aggressively infused soon after the start of the hemorrhage, similar to the actions of a diligent anesthesiologist after an emergency intraope-
ative hemorrhage. The control group received only continued volume support with LR. Hemodynamic measurements were made for 2 h after transfusion in the anesthetized animals and for 2 days of postoperative recovery during which the sheep were monitored in a large-animal intensive care unit.

We hypothesized that the potent volume expansion properties of DCLHb would limit the increase in total Hb concentration compared with transfusion of pRBCs. A key question was to define the hemodynamic consequences resulting from the different properties of DCLHb, pRBCs, and LR in a model of major surgical stress combined with a large hemorrhage treated initially with crystalloid to stabilize cardiovascular function before randomization to treatment group.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston, with adherence to National Institutes of Health Guide for Care and Use of Laboratory Animals [DHHS Publication (NIH) 86-23].

Animals. All RBC transfusions were autologous donations made 8–10 days before the experiment. Nineteen adult me-rino sheep, weighing 24–42 kg (mean 31.1 ± 1.2 kg), were bled 18–22 ml/kg from a percutaneously placed jugular catheter (Insyte-W16G2In IV catheter/needle unit, Becton Dickson Vascular Access, Sandy, UT). This volume of blood was collected to provide a source for the 2 g/kg dose of pRBCs stored in two citrate phosphate dextrose adenine (CPDA) blood collection bags containing 63 ml of citric acid (Teruflex blood bag system; CPDA-1 solution, Terumo, Tokyo, Japan). The DCLHb provided by Baxter Hemoglobin Therapeutics was the same product used in their clinical trials with physical properties previously described (23, 29, 30). In brief, DCLHb is a 10 g/dl solution of purified human Hb with alpha chains cross-linked with bis(3,5-dibromosalicyl)fumarate. Methemoglobin (metHb) content is <5%. We measured it by using a IL-482 cooximeter and found it to be 2.5–4%. The solution is made isotonic at 300 mosmol/kgH2O in a sodium lactate mixture.

Experimental surgery. Sheep were fasted 2 days before the experiment. The surgical placement of vascular lines and a 2-h abdominal surgery were used as an intraoperative model of surgical stress. On the day of the experiment, sheep were sedated with ketamine (10 mg/kg im, Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) for induction of anesthesia. Sheep were surgically prepared in a sterile operating environment, orotracheally intubated (8- or 10-mm-ID cuffed tracheal tube, Mallinckrodt, St. Louis, MO), and mechanically ventilated (Narkomed 2A, North American Drager, Telford, PA) under 1.5–2.5% isoflurane (Abbott Laboratories, Chicago, IL) anesthesia in 50% oxygen. Tidal volume and respiratory rates were initially set at 14 ml/kg and at 15 breaths/min, and, thereafter, respiratory rate was adjusted to maintain an arterial PCO2 between 30 and 35 Torr. This began a 3- to 4-h period of extensive sterile surgery, including placement of vascular catheters, a major abdominal surgery, and abdominal organ manipulation. During the first hour, four vascular catheters were inserted in the right and left femoral arteries and veins to access the abdominal aorta and the inferior vena cava, respectively. On the left side, large femoral catheters were used, fabricated from an intravenous extension set cut down to 24 in. (Baxter Healthcare, Deerfield, IL). On the right side, smaller femoral Intracath 16-gauge 24-in. intravenous catheters (Becton Dickinson) were placed. This allowed simultaneous measurement of arterial pressure, performance of hemorrhage, blood sampling, and infusions. A pulmonary arterial catheter (7-Fr Oximetrix Opticath, Abbott Laboratories) for measurement of mixed venous oxygen saturation (SvO2) and cardiac output (CO) was introduced through the right common jugular vein and positioned in the pulmonary artery. A urinary bladder catheter (14-Fr, Sherwood Medical, St. Louis, MO) was placed via the urethra for monitoring urine flow rates. Intravenous warm LR (Hotline, Smith Industries Medical Systems, Rockland, MA) was infused during the surgery to maintain normal filling pressures and hemodynamics. During surgery, the animals were insulated with cotton blankets, except for the area of surgical exposure, in an attempt to maintain body temperature. Heat lamps were also used, and room temperature was kept at 28°C.

After placement of peripheral catheters, the abdominal surgical procedures were started, which required manipulation of the liver, rumen, and intestines, typically lasting 2.5–3 h. Each animal was placed in the left lateral recum-bency position, and as preparation for sterile surgery the flank was scrubbed for a minimum of 10 min with a surgical scrub solution containing providone-iodine. A subcostal incision was made, and the spleen was surgically removed as part of the experimental surgical stress and to prevent splenic release or uptake of RBCs. This also allowed the use of Hct changes as a means to estimate changes in plasma volume (7). A flow probe was placed on the celiac-superior mesenteric artery root. The subcostal incision was closed in two layers. The animal was then placed in the supine position, and the abdomen was scrubbed with a surgical scrub solution. A medial laparotomy from the xiphoid process to pubis was performed. A 6-Fr catheter fabricated from a pediatric suction tube (Cathmark suction catheter, Smith Industries Medical Systems, Keene, NH) was introduced into the portal vein, and placement was verified by digital palpation of the catheter tip inside the portal vein. Another similar 16-Fr catheter was also placed into the hepatic vein. To accomplish the cannulation, the liver was grasped at its distal margin, and the needle was introduced through the parenchyma at a shallow angle, two-thirds the distance from the diaphragm. During aspiration, the needle was advanced toward the center of the liver until blood was easily pulled into the syringe. The wire was placed through the needle and advanced into the hepatic vein. To verify that the wire was within the hepatic and not the portal vein, it was advanced past the hepatic and into the vena cava and then pulled back to the hepatic vein level. Placement of the catheter was verified by advancing it to a premeasured graduation on the catheter. A tonometer was placed in the terminal ileum. All catheters were exteriorized through the abdominal wall, tunneled subcutaneously, and exteriorized through the skin, and the incisions were sutured closed. Catheters were connected to pressure transducers (Baxter pressure monitoring kit, Baxter Healthcare, Irvine, CA) with continuous flushing devices (normal saline solution with heparin 3 U/ml) connected to a Hewlett-Packard monitor, model 78901-A (Hewlett-Packard, Andover, MA), for continuous hemodynamic monitoring. The abdominal cavity was packed with gauze and loosely closed during the next intraoperative phase of the study. During the remaining intraoperative phase, the anesthetized sheep was sequentially subjected to a baseline period, a large hemorrhage, and resuscitation with LR followed by randomization to one of the three treatment groups for intraoperative anemia as described in detail below.

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Protocol. A key goal of the study, and the focus of this analysis, was to determine the acute and sustained systemic effects of transfusion of DCLHb or pRBCs to correct anemia in sheep subjected to major abdominal surgery and intraoperative hemorrhage. Data from the visceral instrumentation are beyond the focus of the present analysis and are not presented.

After the 3- to 4-h surgical preparation, data collection was started as part of a 3.5-h simulation of a rapid intraoperative hemorrhage and its subsequent treatment. The intraoperative simulation was divided into three periods: a 1-h baseline; a 30-min hemorrhage period with initial treatment of LR only; and a 2-h posthemorrhage period of pRBCs, DCLHb, or a third control group that continued to receive LR only.

Baseline period. Hemodynamic parameters were recorded, and blood samples were taken during a 1-h baseline period. The LR infusion was continued as needed to maintain filling pressure and CO at steady levels during surgery and the baseline period.

Hemorrhage period. After the last baseline measurement, all sheep were submitted to a rapid 10- to 15-min hemorrhage, with bled volume calculated by the following equation to reduce Hb to 5 g/dl

\[ \text{Hemorrhaged volume} = 65 \text{ ml/kg} \times \frac{\text{Hb} - 5}{\text{Hb} \times 0.85} \]

where 65 ml/kg is an estimated blood volume for sheep (33) and initial or prehemorrhage Hb concentration. The value 0.85 is an estimated correction for the rapid hemodilution that occurs during hemorrhage as LR is infused and blood is simultaneously withdrawn. This correction was determined previously in pilot experiments.

All infusions and the 7–8 h of anesthesia were performed by two clinical anesthesiologists (LV, SP) who also participated in protocol design. Specific directions to the anesthesiologists controlling the fluid therapy were to use their clinical judgment and attempt to maintain filling pressure and CO during the surgical preparation. During the hemorrhage, the anesthesiologists were directed to infuse LR to avoid a large emergency hemorrhage. Starting within a few minutes of hemorrhage, LR was aggressively infused and blood simultaneously withdrawn. This correction was determined previously in pilot experiments.

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Transfusion period and treatment groups. Sheep were then randomized to be maintained with LR only (LR group, n = 6) or infused with pRBCs (n = 7) or DCLHb (n = 7). One investigator kept randomization, whereas the anesthesiologists, surgeons, and technicians performing intraoperative care were not informed of the group assignment until shortly before transfusion. This prevented alterations in the preparation or pretreatment care in anticipation of a specific treatment. The Hb dose for the pRBCs and DCLHb groups was the same (2 g/kg of body wt), and both were delivered over the first 30 min of a 2-h intraoperative treatment period.

Postoperative recovery. During the last 30–45 min of anesthesia, all surgical packing was removed, and the abdominal incision was carefully closed using 0-Vicryl (Ethicon, Somerville, NJ) and 0-Prolene (Ethicon). Anesthesia was discontinued, and the sheep were allowed to recover. After each sheep was spontaneously breathing, it was transferred to a large-animal intensive care unit for postoperative care, including pain management and hemodynamic monitoring for 48 h. During postoperative care, the fluid-infusion rates were adjusted to maintain filling pressures and a urine output >1 ml·kg⁻¹·h⁻¹. Buprenorphine (0.3 mg im) was administered during recovery from anesthesia and then every 6–12 h to minimize postsurgical pain. We have learned to recognize behavioral signs when sheep are uncomfortable and assess the need for additional pain medication. These are a lowering of the head, drooping of ears, grinding of teeth, and narrowed eyes.

Measured variables. Data collected included mean arterial pressure (MAP), pulmonary arterial pressure (Ppa), right atrial pressure (RAP), and pulmonary artery occlusion pressure (PAOP), commonly called pulmonary wedge pressure. CO was measured in triplicate by the thermodilution technique, and results were averaged. Blood-gas analysis was performed on both arterial and mixed venous (pulmonary artery) blood samples (System 1302, Instrumentation Laboratory, Lexington, MA). Total Hb, metHb, and percent oxygen saturation were measured with a cooximeter (IL-482, Instrumentation Laboratory) calibrated for human blood. Hct was determined for each blood sample by capillary tube centrifugation.

Calculated variables. Systemic oxygen delivery (DO₂) and systemic oxygen consumption (VO₂) were calculated from the following formulas

\[ \text{DO}_2 = \text{CO} \times \text{CaO}_{2} \]

\[ \text{VO}_2 = \text{CO} \times (\text{CaO}_{2} - \text{CvO}_{2}) \]

where arterial content of oxygen (CaO₂)

\[ = (0.136 \times \text{Hb} \times \text{SaO}_2 + 0.003 \times \text{PaO}_2) \]

and mixed venous content of oxygen (CvO₂)

\[ = (0.136 \times \text{Hb} \times \text{SvO}_2 + 0.003 \times \text{PvO}_2) \]

where SaO₂ is the arterial and oxygen saturation of Hb, and PaO₂ and PvO₂ are the partial pressures of arterial and mixed venous blood, respectively.

Statistical methods. All of the outcomes in this study were continuous measures with approximately normal distributions. We used analysis of variance, followed by Tukey’s Studentized range test, to assess differences between the three treatment groups at each time point. Because the Studentized range test controls the experiment-wise error rate, declarations of statistical significance were set at the alpha level of 0.05. To assess within-group changes across time, for example during the anesthetized period vs. recovery period, we used paired t-tests.

RESULTS

All animals required substantial volume support with LR during the surgical preparation to maintain filling pressure and CO. During the surgical preparation and through the end of the baseline period, the sheep required 185.5 ± 19.7, 175.3 ± 18.9, and 207.3 ± 13.1 ml/kg (means ± SE) of LR for the pRBCs, LR, and DCLHb groups, respectively. This large volume of fluid support likely reflects several factors, including a lengthy major abdominal surgery and the sheep being supine, which is an abnormal position for sheep and can impair venous return. Also, all animals experienced intraoperative hypothermia as body temperatures fell from normal awake values of ~39°C to 36–37°C by the baseline period, with no significant differences between groups.

The experimental groups were very similar at baseline with Hb values of 8–9 g/dl. Figure 1A displays...
Despite infusion of an identical mean dose of Hb (2 g/kg) in the pRBCs and DCLHb groups, the pRBCs group had a higher mean level of blood Hb (8.3 ± 0.5 g/dl) 120 min (T120) after treatment started compared with the DCLHb group’s mean Hb level (7.2 ± 0.4 g/dl). However, this difference was not statistically significant. The LR group had a low Hb level of 5.3 ± 0.3 g/dl, which was virtually unchanged from the hemorrhage level and was significantly lower than the other two groups. During postoperative recovery, all groups exhibited slight decreases in the Hb level; however, at 48 h of postoperative recovery, the Hb levels were still significantly higher in the pRBCs and DCLHb groups, 7.7 ± 0.5 and 6.7 ± 0.2, respectively, compared with the LR group 4.8 ± 0.4 g/dl.

Figure 1B displays the mean values of Hct during the experiment for all three groups. At baseline, groups had similar levels of Hct, ranging from 23 to 26. These values are slightly low for normal healthy sheep (typically 28–32); again, these values reflect the predonation of pRBCs and surgical reparation. Hct levels below normal are not an uncommon finding in surgical patients. After the hemorrhage and LR infusion, the Hct levels fell to a level of 15–17. During treatment, only the pRBCs group showed a rapid and significant increase in Hct to near baseline levels of ~24 during the 120-min posttreatment and only a slightly lower sustained level of 22–23 through the postoperative recovery period. The LR group maintained a stable Hct at the low level of ~15, from the end of hemorrhage through the 48-h postoperative recovery. On the other hand, the DCLHb group showed a significant decrease in the Hct levels to 11.5 ± 0.7 at 30 min after the treatment started. However, Hct returned to the pretreatment hemorrhage levels (15.2 ± 0.5 g/dl) at the first postoperative measurement. This suggests a transient period of plasma volume expansion after the DCLHb infusion that ended early during the postoperative recovery. After 10 h of recovery (R10) and until the end of the experiment, Hct levels recovered slightly to ~16.5 g/dl in the DCLHb group, suggesting a further slight loss of plasma volume.

Figure 2A displays MAP. All three groups show similar curves from baseline until the end of hemorrhage with no significant differences detected between groups. Despite the LR infusions that maintained cardiac filling pressure, at 15 min posthemorrhage MAP decreased ~25 mmHg below baseline. MAP slightly improved ~15 mmHg below baseline before treatment started at 30-min posthemorrhage. After treatment, the DCLHb group displayed a rapid and significant increase in MAP from 75 to 115 mmHg at 30 min after treatment started (T30). The pRBCs and LR groups had slower and smaller increases during the intraoperative period. During postoperative recovery, MAP significantly increased in both the LR and pRBCs groups, such that there were few significant differences between the three groups. During postoperative recovery, MAP was sustained at 100–120 mmHg with the highest level apparent in the DCLHb group; however, this difference was not significant.
between groups and no apparent fall after hemorrhage per experimental design because of the administered LR. However, after treatment the DCLHb group showed a significantly large transient increase of ~15 mmHg at T30, which subsided to 13 and 7 mmHg at T60 and T120, respectively. The pRBCs and LR groups maintained baseline levels of RAP after treatment. During postoperative recovery, all groups maintained near-baseline levels with no statistically significant differences between groups.

The PAOP is displayed in Fig. 3B and exhibits patterns similar to RAP for each group. The peak PAOP in the DCLHb group was measured at T30, with a value of 20 mmHg. From this point on PAOP values declined over the next 2 h. During postoperative recovery, the mean PAOP of each group were not significantly different from each other, although the pRBCs and especially the DCLHb groups exhibited trends to increase over time.

Figure 4A displays CO expressed as percent of baseline level (l/min). Baseline CO values were 4.5 ± 0.4,
5.2 ± 0.6, and 5.2 ± 0.6 in the pRBCs, LR, and DCLHb groups, respectively. On a per weight unit basis sheep have higher COs than humans, which results from their relatively high normal heart rates and large hearts. All three groups showed little change in CO and no significant group differences during baseline, hemorrhage, or during treatment. However, with postoperative recovery CO significantly increased in both the LR and pRBCs groups but not in the DCLHb group. The LR group showed the highest CO level (150–160% of baseline levels) during postoperative recovery time. The DCLHb group had the lowest CO [80% of baseline level at 5 h into the postoperative recovery period (R5)], whereas the pRBCs group had an intermediate position (130% of baseline). Base excess data show no significant differences over time or between groups. Group differences: *P < 0.05 DCLHb vs. LR; ‡P < 0.05 DCLHb vs. pRBCs.

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There were no significant differences between treatment groups for either heart rate or stroke volume. Mean baseline heart rates were ~110 beats/min, and mean baseline stroke volumes were ~45 ml. Heart rates and stroke volumes did not change significantly during the intraoperative phase. DCLHb infusion resulted in a lowered heart rate for the first hour post-transfusion, presumably because of transient hypertension. During recovery, heart rate displayed increased variance in all groups with a not significant trend of an increase in the LR group and a decrease in the pRBCs group. Stroke volume increased with postoperative recovery in all three groups with no group differences as CO increased.

Figure 4B displays extracellular base excess as calculated from the blood gases. Baseline base excess shown in Fig. 4 was approximately zero and reflects the effects of surgical stress and anesthesia. Healthy conscious sheep have a base excess level of +3 to +6, which reflects their normal pH of 7.45–7.55. All three
groups exhibited a slight decrease in base excess, 1–2 meq below their baseline intraoperative levels, during the hemorrhage. During recovery, all groups recovered to above baseline intraoperative levels of base excess. Differences between groups and difference of groups over time were not significant.

Figure 5 displays the calculated DO2 and VO2 of oxygen. The DO2 had a decrease during hemorrhage of ~40% in all groups, directly proportional to the reduced Hct. During the 2 h intraoperative treatment period, DO2 in the pRBCs (P < 0.05) and DCLHb (P > 0.05) groups steadily improved after transfusion, but values remained slightly decreased ~10–15% below baseline, whereas the LR group exhibited no improvement compared with the LR DO2 during the hemorrhage. During the postoperative recovery, the DO2 of the pRBCs group significantly increased and was the highest DO2 of any group and near maintained until the experiment ended.

SaO2, % increased and was the highest DO2 of any group and near maintained until the experiment ended. This increase was mainly increased approximately twofold from the recovery, the DO2 of the pRBCs group significantly increased, but the DCLHb group did not have a statistically significant change. Group differences after the intraoperative treatment were only statistically significant at R5, with the DO2 for the pRBCs group being significantly greater than the DCLHb group.

Figure 5B displays VO2, which shows no apparent change or significant group differences during baseline hemorrhage and treatment, maintaining the anesthetized baseline levels of 100 ml/min for all groups. During the awake and recovery phase, VO2 levels significantly increased approximately twofold from the anesthetized baseline levels. This increase was maintained until the experiment ended.

Table 1 displays PaO2, PVO2, SaO2, MetHb, and CaO2.

Table 1. PaO2, PVO2, SaO2, MetHb, and CaO2  

<table>
<thead>
<tr>
<th>PaO2, Torr</th>
<th>BL</th>
<th>H30</th>
<th>T120</th>
<th>R24</th>
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<tr>
<td>RBCs</td>
<td>308 ± 3</td>
<td>319 ± 32</td>
<td>258 ± 36</td>
<td>92 ± 1.4</td>
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<tr>
<td>LR</td>
<td>299 ± 15</td>
<td>283 ± 17</td>
<td>266 ± 23</td>
<td>95 ± 3.2</td>
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<td>DCLHb</td>
<td>291 ± 13</td>
<td>284 ± 13</td>
<td>260 ± 27</td>
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<td>PVO2, Torr</td>
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<td>65 ± 2.9</td>
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<td>LR</td>
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<td>DCLHb</td>
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<td>SaO2, %</td>
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<tr>
<td>MetHb in whole blood, %</td>
<td>pRBCs</td>
<td>0.9 ± 0.1</td>
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<tr>
<td>DCLHb</td>
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<td>CaO2, ml/dl</td>
<td>pRBCs</td>
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<td>8.9 ± 0.6</td>
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</table>

Values are means ± SE. PaO2, arterial partial pressure of oxygen; PVO2, mixed venous partial pressure of oxygen; SaO2, arterial oxygen saturation; MetHb, methemoglobin; CaO2, arterial oxygen content; BL, baseline period; H30, 30-min hemorrhage; T120, 120 min of intraoperative transfusion; R24, 24-h after postoperative recovery; RBCs, red blood cells; pRBCs, packed RBCs; LR, lactated Ringer solution; DCLHb, diaspirin cross-linked hemoglobin. Group differences:

- P < 0.05 DCLHb vs. LR;
- †P < 0.05 pRBCs vs. LR;
- ‡P < 0.05 DCLHb vs. pRBCs.

DISCUSSION

Animals were subjected to a major surgical stress in that they were anesthetized 7–8 h and had two abdominal incisions with major procedures performed and...
three peripheral incisions for catheter placement. Additional cardiovascular stress was likely caused by the sheep being in the supine position for most of the intraoperative period and becoming hypothermic despite efforts to maintain body temperature. Such stresses and the resultant volume requirements are not representative of a simple or even a complex elective surgical procedure that proceeds routinely. In the authors’ opinion, these changes are more representative of a difficult elective surgical case such as esophagectomy or spine surgery with complications. Perhaps the combined hypothermia and surgical stress we imposed are the most representative of physiological challenges in emergency trauma followed by surgery with significant blood loss.

We hypothesized that the potent volume expansion properties of DCLHb would dilute out the Hb concentration in blood compared with pRBCs. The two specific and clinically relevant questions we hoped to address in this study were 1) How does treatment with an equivalent Hb dose of either pRBCs or DCLHb compare in a model of surgical stress, hemorrhage, and induced anemia with respect to the intraoperative and postoperative hemodynamics? and 2) How are oxygen content, delivery, and consumption affected by these two treatments?

**Blood volume expansion.** The Hct and Hb changed in the same direction, both in the pRBCs and LR groups. The LR group maintained a steady reduced level (anemia) in both Hct and Hb, whereas the pRBCs group restored both Hb and Hct to near baseline. On the other hand, the DCLHb group caused a rise in Hb and a fall in Hct; these changes are due to a combination of the plasma volume expansion (7, 14) and plasma concentration of Hb after DCLHb infusion.

It is possible to estimate the initial blood volume expansion due to the infusion of 20 ml/kg DCLHb. We can assume that, during the 30-min period of hemorrhage and the LR infusion, normovolemic hemodilution was achieved because filling pressures were maintained. The normal blood volume for sheep is 65 ml/kg. If Hct were decreased from 15.5 to 11.5 g/dl, then the new calculated blood volume due to plasma volume expansion would be 88 ml/kg. This represents a ~23 ml/kg expansion or 115% of the 20 ml/kg infusion. The ability of DCLHb to expand blood volume more than infused volume has been reported to be ~130% of infused volume in a sheep model of simple hypovolemia (7). A lesser expansion in the present study may reflect a greater transvascular loss of fluid, perhaps because of a capillary leak induced by the surgical trauma. Urine output levels were similar in all three groups during the intraoperative period.

Enhanced volume expansion properties of RBC substitutes and volume expanders may be considered a desirable feature. Improved volume expansion is the main rationale for the use of colloids over crystalloids. Enhanced volume expansion largely explains the effectiveness of small volume hypertonic formulations (31, 32). However, enhanced volume expansion is also a limitation for a RBC substitute. Plasma volume expansion limits the ability to improve the oxygen content of blood because both the preinfusion Hct is reduced and the concentration of infused Hb itself is diluted. Indeed, Hct was significantly reduced from 15.5 to 11.6 in the DCLHb group. Furthermore, 2 h after an equal-Hb dose of 2 g/kg of DCLHb or pRBCs, the resultant concentration of total Hb increased 3.2 ± 0.3 g/dl after pRBCs and only 1.7 ± 0.2 g/dl after DCLHb. These data support our hypothesis that DCLHb potent volume expansion properties contribute to a lower Hb concentration. This higher mean of the Hb concentration contributed to the statistically significant increase in DO₂ after treatment and again after recovery in the pRBCs group, whereas the lower mean Hb in the DCLHb contributed to the lack of significant increase with DCLHb at these time points. An ideal blood substitute formulation might have a low to normal colloid osmotic pressure with a high Hb concentration. For reasons not entirely clear to us, few RBC substitutes in development have such properties.

**Hemodynamics.** As stated in MATERIALS AND METHODS, the directions to the anesthesiologist during the hemorrhage were to aggressively correct the volume deficit by infusing LR to restore and maintain filling pressures and CO. This goal was accomplished as the RAP, PAOP, and CO did not fall significantly after hemorrhage. However, despite the infusions, MAP was reduced 15–25 mmHg and Ppa was reduced 2–3 mmHg. This may reflect the reduced viscosity of whole blood due to hemodilution.

The 30-min hemorrhage period and LR volume infusion could be described as acute normovolemic hemodilution, which is often reported to be associated with increased CO instead of the unchanged CO and reduced arterial pressure we observed. However, the effects of anesthesia and surgical stress may blunt the ability of CO to increase during hemodilution (24).

More importantly, CO levels significantly increased in both the LR and pRBCs groups during postoperative recovery but not in the DCLHb group. The DCLHb group maintained CO levels during recovery similar to intraoperative levels with one prominent low point at R5. Infusion of several Hb-based RBC substitutes have been shown to cause lower CO levels than in the control groups treated with traditional volume expanders (7, 14, 35). For example, Fischer et al. (7) found that CO levels increased similarly in both normovolemic and hemorrhaged sheep after infusions of either a large volume of crystalloid (60 ml/kg LR) or a smaller volume of colloid (20 ml/kg human albumin). On the other hand, 20 ml/kg DCLHb expanded blood volume more than either a large volume of LR or equal volumes of albumin. Furthermore, DCLHb increased RAP more, but caused only a modest increase in CO in hemorrhaged sheep and reduced CO in normovolemic sheep.

In the present study, the DCLHb treatment expanded blood volume better than the pRBCs or the LR on the basis of greater reductions in Hct and higher filling pressures. Despite increased blood volume after DCLHb infusion, CO levels were similar in all three
groups immediately after the intraoperative treatment. Also, CO levels increased in the postoperative recovery period, both in the LR and pRBCs groups, but did not in the DCLHb group. Increased metabolism and CO levels are an appropriate and expected response during postoperative warming and recovery from anesthesia. We suggest that DCLHb may have impaired CO because of either an increased afterload from vasoconstriction or a direct cardiac systolic or diastolic dysfunction or a combination of these factors. Such effects may be a limitation of DCLHb and perhaps of other first-generation RBC substitutes. The effects of α-α-Hb (US Army), which is the same molecule as DCLHb (Baxter) suspended in a different buffer, caused coronary vasoconstriction (18). Decreased CO levels have also been reported after infusion with a bovine Hb-based RBC substitute (14, 35). On the other hand, studies have shown an increase in total coronary blood flow after DCLHb treatment (11), and no deleterious cardiac effects have been reported in clinical trials (17).

It is interesting to speculate that an impaired ability to increase CO may be related to Hb binding of nitric oxide (NO). Comparisons between NO binding, Hb solutions and new second-generation non-NO-binding RBC substitutes are needed to compare their effects on CO and cardiac function in clinically relevant animal models. It has been suggested that a reduced CO after infusion of a RBC substitute is an “appropriate” response, as there is less need for increased CO and work because both blood oxygen content and DO2 are increased (7). However, pRBCs also increase oxygen content, whereas other volume expanders such as LR and albumin generally increase both CO and DO2. Clearly, DCLHb and some of the other first-generation Hb-based RBC substitutes are unique volume expanders, as they appear to have a specific effect to inhibit CO despite significant volume expansion (7, 14, 35).

**Oxygen content and delivery.** CaO2 was significantly higher after the pRBCs transfusion than after the DCLHb transfusion for two reasons. Mean Hb was lower, albeit not significantly, and SaO2 was significantly lower after the Hb DCLHb treatment because of an increased metHb. After intraoperative treatment, DO2 was only significantly increased in the RBC group. After recovery, DO2 was increased in both the LR and the pRBCs group but not in the DCLHb group. Despite these differences in the group responses, over time there was a treatment effect for DO2 at only one time point, R5, when DO2 was significantly higher for the pRBCs group vs. the DCLHb group. Despite differences in DO2, there were no apparent treatment effects on either VO2 or base excess. Thus it could be viewed that the DCLHb treatment was as physiologically effective as the other two treatments. On the other hand, we have no data to suggest that the DCLHb treatment offered any physiological benefit compared with the pRBCs treatment in this animal model. Likewise, it can be viewed that there was no apparent advantage in the pRBCs group compared with the LR group, given that all survived in both groups and VO2 and base excess were not significantly different. It may require a greater hemorrhage and/or a more severe anemia to demonstrate a significant difference and need for transfusion. Our study may be one demonstration of the difficulties involved in showing a physiological advantage to a RBC substitute. As with different volume expanders and the use of pulmonary artery catheters, it may be extremely difficult to prove a statistically significant comparative clinical benefit of a RBC substitute vs. human blood of pRBCs.

A limitation of our calculations is that DO2 and VO2 values are calculated from SaO2 and SV02 measurements on an IL-482 using human settings. The IL-482 oximeter was not calibrated, nor does the company provide a setting, for sheep blood. The SaO2 and DO2 values should be accurate, because at high saturations there is little difference between the human and sheep oxygen dissociation curves. The SV02 values are likely to be higher than measured because of a rightward shift in the oxygen dissociation curve of sheep blood compared with human blood (6, 26). This would tend to decrease DO2 values. However, because SV02 and PV02 values were not significantly different between the groups, the correction should not affect the comparative results. Another complication is that DCLHb also has a unique dissociation curve. The oxygen dissociation curve has not been published for DCLHb but has been published for the similar α-α-Hb (34). Nevertheless, oxygen saturation of patients administered DCLHb is typically measured with standard human cooximeter settings on a clinical cooximeter. Future cooximeters may require settings to allow for measuring oxygen saturation with different mixtures of RBC-Hb and blood substitute-Hb.

**Clinical implications.** Most of the early animal studies suggested some physiological benefit to the DCLHb treatments (4, 5, 7, 25). Furthermore, early clinical studies suggested that the DCLHb treatment was clinically safe, delivered and unloaded oxygen, and could reduce RBC requirements in surgical operations (17, 20, 21). However, the commercial development of DCLHb was abruptly halted in 1998. An interim safety analysis of a Food and Drug Administration phase III, multicenter US emergency room study of DCLHb treatment of severe trauma showed an increased mortality in the DCLHb groups compared with the control patients receiving standard care of crystalloid and pRBCs treatment. An extensive analysis of the trial data has failed to provide a satisfactory explanation of the increased mortality (29). Nor do the present study and our data provide a satisfactory explanation for the one death and significant morbidity (an inability to stand) that was observed in another sheep of the DCLHb group. There is little that can be concluded statistically from our mortality and morbidity data. Such outcomes may or may not have been related to the treatment.

Although there were clear physiological differences between the treatments, our data do not prove a deleterious outcome effect of DCLHb. However, our data suggest at least a potential limitation of DCLHb, which
occasionally may affect clinical outcome when combined with major physiological stress conditions. We suggest that the US trauma trial of DCLHb may have exhibited such deleterious effects when patients suffering from severe traumatic injuries and hemorrhage were administered DCLHb infusion. Furthermore, we suggest that this same limitation may have accounted for the mortality in our study as we used an animal model of severe surgical stress and hemorrhage.

We speculate on two possible theories for DCLHb treatment limitation: 1) large-volume infusions of LR combined with the DCLHb treatment caused greater cardiovascular stress due to combined hypervolemia and vasoconstriction than with either treatment alone, and 2) DCLHb treatment impairs the ability of the heart to increase CO under conditions of increased preload or increased demands for greater D\(\dot{O}_2\).

The combinations of large-volume LR and DCLHb can cause hypervolemia and aggravate the increased afterload due to vasoconstriction and the increased preload due to hypervolemia and perhaps also due to a direct cardiac effect. Nearly all preclinical animal studies and clinical trials evaluated infusions of DCLHb alone vs. various control volume expanders. There have been few studies in which large-volume LR was infused along with DCLHb as done in the present study and also reported for most patients in the US trauma trial of DCLHb. Many, if not most, of the severe trauma patients in the DCLHb trauma trial received substantial volumes of LR, either in the field or the emergency room before receiving DCLHb. Interestingly, in a European prehospital trauma trial in which DCLHb was used alone as the initial treatment and without LR an increased mortality was not apparent in the DCLHb groups (29).

We speculate that if the myocardium has already been compromised, because of either preexisting disease or the effects of trauma and shock, then the added stress of hypervolemia, increased afterload, and increased cardiac work could lead to an immediate cardiac failure or a cardiac depression, which subsequently manifests during recovery. We recently reported that combinations of large volume of LR and DCLHb aggravate the systemic and particularly the pulmonary hypertension of DCLHb (1–3). In the present study, systemic pressures did not increase to levels of clinical concern, but both left and right filling pressures and the pulmonary pressure were exceedingly high particularly during the first 30–60 min after administration of DCLHb. Trauma patients are not routinely monitored for either filling pressure or pulmonary pressure in the emergency room. Excessive filling or pulmonary pressures could have occurred in some of the patients in the DCLHb trauma trials.

Despite volume expansion, DCLHb and other RBC substitutes have a consistent record of causing only mild increases in CO levels. Fischer et al. (7) found that CO increased less after DCLHb than with either equal volumes of albumin or large volumes of LR despite greater volume expansion with DCLHb. In normovolemia, DCLHb and other RBC substitutes often cause decreases in CO (14, 35). We suggest the possibility that DCLHb limits the ability of the heart to respond appropriately to increases in either preload or metabolic demands. The overall safety and efficacy record of DCLHb, in both animal and human studies, indicates that such a limitation was rarely life threatening. However, an inability to increase CO levels appropriately has been correlated with increased morbidity in critically ill trauma patients after surgical stress or accident (27). In the present study, CO was increased during postoperative recovery as metabolism increased in both the LR and the pRBCs groups but not in the DCLHb group.

In summary, we have compared infusions of equal Hb doses of the DCLHb treatment and the pRBCs treatment for hemorrhagic anemia with a LR control group in an animal model of major surgical stress. There were no definitive deleterious or beneficial effects established with any of the treatments. Furthermore, the data suggest significant physiological stress, hypervolemia, and elevated preload and afterload pressure with DCLHb treatment. These combined effects may cause an impairment of the ability to augment CO when large volumes of LR are administered before DCLHb. Such physiological stresses and cardiac impairments may account in part for the deleterious mortality outcomes reported in the US multicenter trauma trial of DCLHb.

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